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(54) Title: RETROVIRAL PROTEASE INHIBITOR COMBINATIONS

(57) Abstract

The present invention is directed to a method for the treatment of mammalian retrovirus infections, such as HIV, using combinations of retroviral protease inhibitors which are effective in preventing the replication of the retroviruses *in vitro* or *in vivo*. This invention, in particular, relates to protease inhibitor compounds used in combination therapy with other protease inhibitor compounds. This invention also relates to combination therapy with a combination of protease inhibitors and antiviral agents other than protease inhibitors.

RETROVIRAL PROTEASE INHIBITOR COMBINATIONS

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a method for the treatment of mammalian retrovirus infections, such as human immunodeficiency virus (HIV), using combinations of retroviral protease inhibitors which are effective in preventing the replication of mammalian retroviruses, like HIV, in vitro and in vivo. This invention, in particular, relates to protease inhibitor compounds used in combination therapy with other protease inhibitor compounds.

2. Related Art

During the replication cycle of retroviruses, gag and gag-pol gene transcription products are translated as proteins. The proteins are subsequently processed by a virally encoded protease (or proteinase) to yield viral enzymes and structural proteins of the virus core. Most commonly, the gag precursor proteins are processed into the core proteins and the pol precursor proteins are processed into the viral enzymes, e.g., reverse transcriptase and retroviral protease. It has been shown that correct processing of the precursor proteins by the retroviral protease is necessary for assembly of infectious virons. For example, it has been shown that frameshift mutations in the protease region of the pol gene of HIV prevents processing of the gag precursor protein. It has also been shown through site-directed mutagenesis of an aspartic acid residue in the HIV protease active site that processing of the gag precursor protein is prevented. Thus, attempts have

BRIEF DESCRIPTION OF THE INVENTION

The present invention is directed to a method for the treatment of mammalian retrovirus infections, such as human immunodeficiency virus (HIV), using combinations of retroviral protease inhibitors which are effective in preventing the replication of the retroviruses in vitro or in vivo. This invention, in particular, relates to protease inhibitor compounds used in combination therapy with other protease inhibitor compounds. Further, this combination may also be used in combination with other anti-viral agents.

DETAILED DESCRIPTION OF THE INVENTION

Retroviral protease is a critical enzyme in the retroviral replication process. Propagation of a retrovirus, such as HIV, can be impeded by exposing the virus to a retroviral protease inhibitor. However, with prolonged exposure of the retrovirus to the protease inhibitor, the variant retroviruses can be selected such that a new predominant strain of retrovirus resistant to the protease inhibitor emerges. The new predominant strain of retrovirus can produce a protease which is no longer inhibited or more frequently is insufficiently inhibited by the protease inhibitor and can freely propagate even in the presence of the protease inhibitor unless the concentration of the inhibitor is substantially increased. The present invention provides a method for overcoming the development of retroviral strains which are resistant to a retroviral protease inhibitor.

The present method provides for the administration to a mammal, such as a human, monkey, cat and the like, of an effective amount of at least two retroviral protease inhibitors. The administration may be accomplished by co-administration of at least two retroviral protease

phenotype may be divided into no resistance, low level resistance (less than about 10 fold shift in EC_{50} or EC_{90}), intermediate level resistance (about 10 to about 100 fold shift in EC_{50} or EC_{90}) or high level resistance (greater than about 100 fold shift in EC_{50} or EC_{90}). It is anticipated that drug resistance will correlate with a reduced effect on patient viral load when the achievable in vivo inhibitor concentrations have a reduced protease inhibition effect on the resistant virus. Thus the more preferred combinations of protease inhibitors will be those that exhibit minimal cross-resistance profiles (i.e., preferably, not more than intermediate level resistance; more preferably, not more than low level resistance; and most preferably, no resistance) and maximal intrinsic potency for wild-type and/or resistant viruses selected against another inhibitor. For example, preferred compounds for use in combination with a first compound will preferably be effective against strains of virus which are intermediate level, more preferably high level, resistant to the first compound. The pharmacology and toxicology of each inhibitor and combination are also factors in the selection of inhibitors for combination therapy.

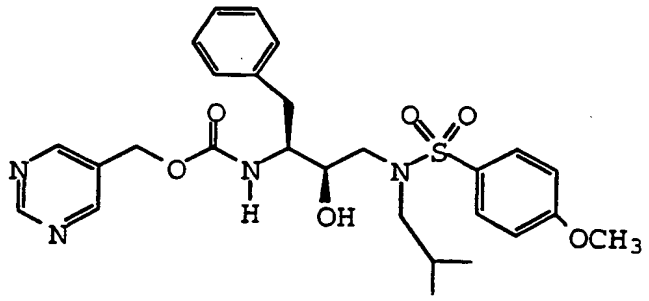
More preferably, retroviral protease inhibitors are chosen when at least one viral resistant strain to a first retroviral protease inhibitor and at least one viral resistant strain to a second retroviral protease inhibitor having different amino acid substitutions in the protease peptide sequence that affect the same substrate binding site region of the protease and contributes to the observed inhibitor resistance. Thus the number of possible amino acid substitutions that may occur in the same site in the protease are limited. This is particularly true when the site is critical to activity, effectiveness and/or stability of the enzyme.

(filed March 2, 1994), 07/886,556 (filed May 20, 1992), 07/886,663 (filed May 20, 1992), 07/886,531 (filed May 20, 1992), 08/148,817 (filed November 8, 1993), 08/886,700 (filed May 21, 1992) and 07/998,187 (filed December 29, 1992) and PCT Patent Applications Nos. PCT/US93/10552 (filed October 29, 1993), PCT/US93/10460 (filed October 29, 1993) and PCT/US93/10461 (filed October 29, 1993), each of which are incorporated herein by reference in their entirety. Additional retroviral protease inhibitors which are suitable for use in the present method include, but are not limited to, the protease inhibitors disclosed and described in US Patent 5,157,041; EP 346,847; US Patent Application Serial No. 07/883,825 (filed May 15, 1992); WO 93/09096; Tet. Lett. 35:673-676 (1994); Proc. Natl. Acad. Sci. USA, 91: 4096-4100 (1994); Y. N. Wong et al., Biopharm. & Drug Dispos. 15:535-544 (1994); M.L. West and D. P. Fairlie, Trends Pharmacol. Sci. 16:67-75 (1995); and S. Thaisrivongs, "HIV Protease Inhibitors", Ann. Reports Med. Chem., Vol. 29, Chap. 14, pp. 133-144 (1994) (Academic Press, J. Bristol, Ed.), each of which is incorporated herein by reference in their entirety.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are not intended to provide an exhaustive description of all possible compound combinations but merely to provide examples of drug combinations that are anticipated to be effective. Similar testing of these and other protease inhibitors using resistant viral isolates, not limited to those listed below, can help identify suitable drug combinations. Therefore, the following preferred specific embodiments are to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

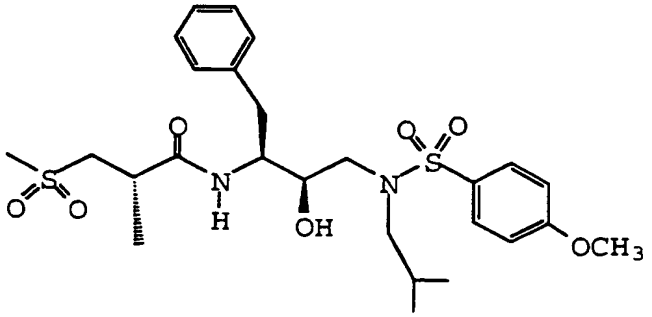
owned and co-pending US Patent Applications Serial Nos. 08/109,787 (filed August 20, 1993), Attorney docket No. 2766/1 co-filed with the present application, and 08/156,498 (filed November 23, 1993, all three incorporated herein by reference in their entirety.

Example 3

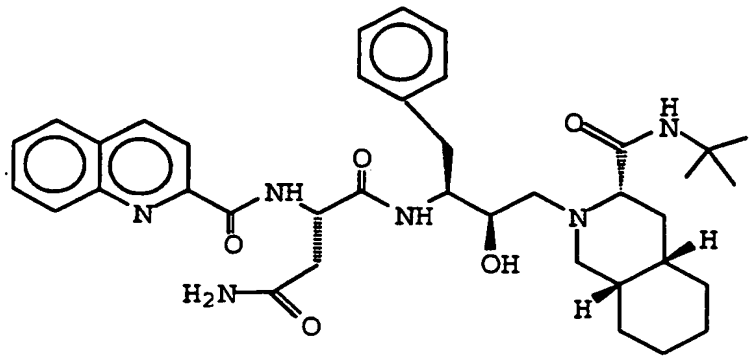


[2R-hydroxy-3-[[(4-methoxyphenyl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]carbamic acid 5-pyrimidylmethyl ester can be prepared according to the methods disclosed in co-owned and co-pending US Patent Applications Serial Nos. 08/110,911 (filed August 24, 1993) and 08/156,498 (filed November 23, 1993), both incorporated herein by reference in their entirety.

Example 4

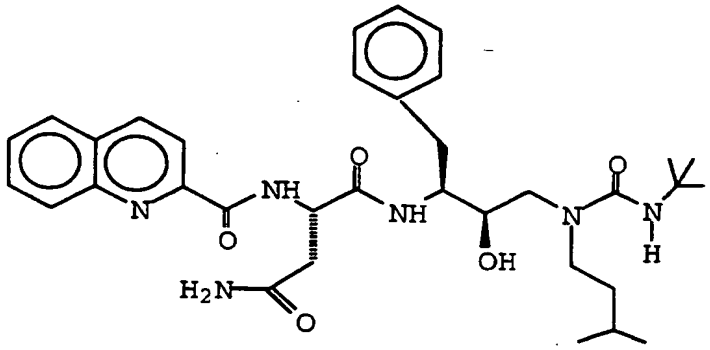


Example 6 - Saquinavir



N-tert-Butyl decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparaginyl]amino]butyl]-(4aR,8aS)-isoquinoline-3(S)-carboxamide (Ro 31-8959) can be prepared according to the methods disclosed in US Patent 5,157,041, incorporated herein by reference in its entirety.

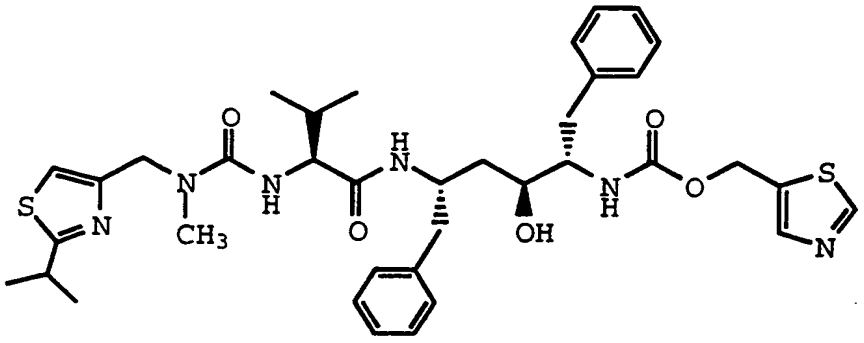
Example 7



[1S-[1R*(R*),2S*]]-N^1-[3-[[[(1,1-dimethylethyl)amino]carbonyl](3-methylbutyl)amino]-2-hydroxy-1-(phenylmethyl)propyl]-2-[(2-quinolinylcarbonyl)amino]-butanediamide can be prepared according to the methods disclosed in co-owned and co-pending US Patent Applications Serial Nos. 08/152,934 (filed November 15, 1993) and

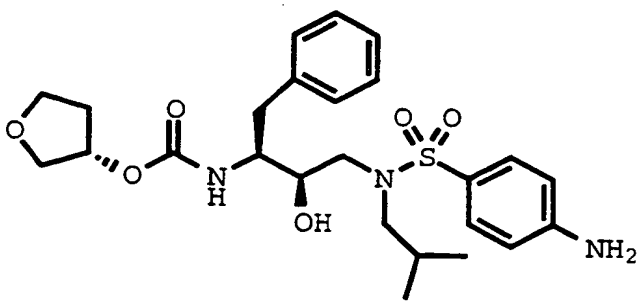
(2S,3R,4S,5S)-2,5-Bis-[N-[N-[[N-methyl-N-(2-pyridinylmethyl)amino]carbonyl]valinyl]amino]-3,4-dihydroxy-1,6-diphenylhexane (A-77003) can be prepared according to the methods disclosed in J. Med. Chem. 36:320-330 (1993), which is incorporated herein by reference in its entirety.

Example 10 - ritonavir

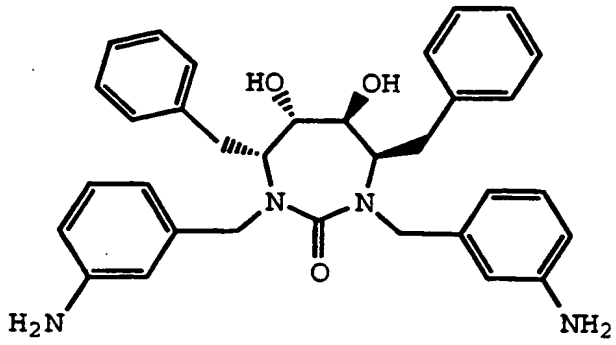


(2S,3S,5S)-5-[N-[N-[N-methyl-N-[(2-isopropyl-4-thiazolyl)methyl]amino]carbonyl]valinyl]amino]-2-[N-[(5-thiazolyl)methoxycarbonyl]amino]-3-hydroxy-1,6-diphenylhexane (A-84538, ABT-538) can be prepared according to the methods disclosed in PCT Patent Application Serial No. WO 94/14436 (filed December 16, 1993), which is incorporated herein by reference in its entirety.

Example 11

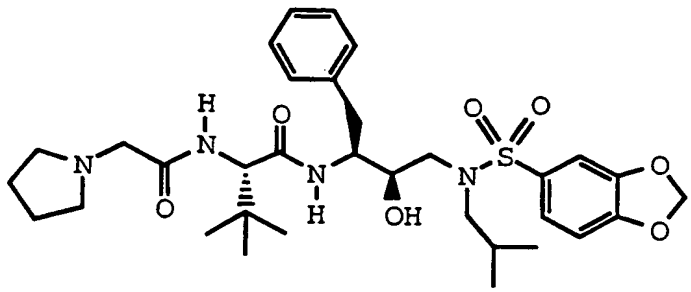


Example 13



[4R-(4α,5α,6ß,7ß)]-1,3-bis[(3-aminophenyl)methyl]hexahydro-5,6-dihydroxy-4,7-bis(phenylmethyl)-2H-1,3-diazepin-2-one (DMP-450, XM-412) can be prepared according to the methods disclosed in PCT Patent Application WO 93/07128, incorporated herein by reference in its entirety. In particular, 3-nitrophenylmethyl halide, such as 3-nitrophenylmethylchloride or bromide, is reacted with the hydroxy protected derivative of [4R-(4α5α,6ß,7ß)]-hexahydro-5,6-dihydroxy-4,7-bis(phenylmethyl)-2H-1,3-diazepin-2-one followed by deprotection of the hydroxy groups (see WO 93/07128) and reduction of the nitro groups to the amino groups. Such reductions can be accomplished using standard procedures well known to those skilled in the art.

Example 14



10 minutes. The layers were separated and the organic layer was washed again twice with water (5l). The organic layer was dried with magnesium sulfate (500g) and filtered to remove the drying agent. The solvent was removed under vacuum at 50°C. The resulting warm liquid was allowed to cool at which time a solid began to form. After one hour, the solid was washed with hexane (400 mL), filtered and dried to provide the desired sulfonyl chloride (2823g). The hexane wash was concentrated and the resulting solid washed with 400 mL hexane to provide additional sulfonyl chloride (464g). The total yield was 3287g (95.5% based upon 1,3-benzodioxole).

Part B: Preparation of 2S-[Bis(phenylmethyl)amino]-3-phenylpropanol

METHOD 1: 2S-[Bis(phenylmethyl)amino]-3-phenylpropanol from the DIBAL Reduction of N,N-bis(phenylmethyl)-L-Phenylalanine phenylmethyl ester

Step 1: A solution of L-phenylalanine (50.0 g, 0.302 mol), sodium hydroxide (24.2 g, 0.605 mol) and potassium carbonate (83.6 g, 0.605 mol) in water (500 mL) was heated to 97°C. Benzyl bromide (108.5 mL, 0.605 mol) was then slowly added (addition time - 25 min). The mixture was stirred at 97°C for 30 minutes under a nitrogen atmosphere. The solution was cooled to room temperature and extracted with toluene (2 x 250 mL). The combined organic layers were washed with water and brine, dried over magnesium sulfate, filtered and concentrated to an oil. N,N-bis(phenylmethyl)-L-phenylalanine phenylmethyl ester can be purified by column chromatography (silica gel, 15% ethyl acetate/hexane). Usually the product is pure enough to be used directly in the next step without further purification. EIMS: m/z 434 (M-1).

L-phenylalaninol (176.6 g, 1.168 mol) was added to a stirred solution of potassium carbonate (484.6 g, 3.506 mol) in 710 mL of water. The mixture was heated to 65°C under a nitrogen atmosphere. A solution of benzyl bromide (400 g, 2.339 mol) in 3A ethanol (305 mL) was added at a rate that maintained the temperature between 60-68°C. The biphasic solution was stirred at 65°C for 55 min and then allowed to cool to 10°C with vigorous stirring. The oily product solidified into small granules. The product was diluted with 2.0 L of tap water and stirred for 5 minutes to dissolve the inorganic by products. The product was isolated by filtration under reduced pressure and washed with water until the pH is 7. The crude product obtained was recrystallized from 1.1 L of ethyl acetate/heptane (1:10). The product was isolated by filtration (at -8°C), washed with 1.6 L of cold (-10°C) ethyl acetate/heptane (1:10) and air-dried to give 339 g (88% yield) of 2S-[Bis(phenylmethyl)amino]-3-phenylpropanol, Mp = 71.5-73.0°C.

Part C: Preparation of 2S-[Bis(phenylmethyl)amino]-3-phenylpropanaldehyde

METHOD 1: 2S-[Bis(phenylmethyl)amino]-3-phenylpropanol (200 g, 0.604 mol) was dissolved in triethylamine (300 mL, 2.15 mol). The mixture was cooled to 12°C and a solution of sulfur trioxide/pyridine complex (380 g, 2.39 mol) in DMSO (1.6 L) was added at a rate to maintain the temperature between 8-17°C. The solution was stirred at ambient temperature under a nitrogen atmosphere for 1.5 hour. The reaction mixture was cooled with ice water and quenched with 1.6 L of cold water (10-15°C) over 45 minutes. The resultant solution was extracted with ethyl acetate (2.0 L), washed with 5% citric acid (2.0 L) and brine (2.2 L), dried over $MgSO_4$ (280 g) and filtered. The solvent was removed in vacuo and then dried under vacuum to give 198.8 g of 2S-

of silica gel. The filtrate was concentrated under reduced pressure to give a product containing approximately 50% of 2S-[bis(phenylmethyl)amino]-3-phenylpropanaldehyde as a pale yellow oil.

METHOD 4: To a solution of 1.0 g (3.02 mmoles) of 2S-[bis(phenylmethyl)amino]-3-phenylpropanol in 9.0 mL of toluene was added 4.69 mg (0.03 mmoles) of 2,2,6,6-tetramethyl-1-piperidinyloxy, free radical (TEMPO), 0.32g(3.11 mmoles) of sodium bromide, 9.0 mL of ethyl acetate and 1.5 mL of water. The mixture was cooled to 0°C and an aqueous solution of 2.87 mL of 5% household bleach containing 0.735 g (8.75 mmoles) of sodium bicarbonate and 8.53 mL of water was added slowly over 25 minutes. The mixture was stirred at 0°C for 60 minutes. Two more additions (1.44 mL each) of bleach was added followed by stirring for 10 minutes. The aqueous layer was extracted twice with 20 mL of ethyl acetate. The combined organic layer was washed with 4.0 mL of a solution containing 25 mg of potassium iodide and water (4.0 mL), 20 mL of 10% aqueous sodium thiosulfate solution and then brine solution. The organic solution was dried over magnesium sulfate, filtered and concentrated under reduced pressure to give 1.34g of crude oil containing a small amount of the desired product aldehyde, 2S-[bis(phenylmethyl)amino]-3-phenyl propanaldehyde.

Part D: Preparation of N,N-dibenzyl-3(S)-amino-1,2-(S)-epoxy-4-phenylbutane

METHOD 1: A solution of 2S-[Bis(phenylmethyl)amino]-3-phenylpropanaldehyde (191.7 g, 0.58 mol) and chloroiodomethane (56.4 mL, 0.77 mol) in tetrahydrofuran (1.8 L) was cooled to -30 to -35°C in a stainless steel reactor under a nitrogen atmosphere. A solution of n-butyl

A 1.6 M solution of n-butyl lithium in hexane (25 ml, 0.040 mol) was then added at a rate to maintain the temperature at -75°C. After the first addition, additional chloroiodomethane (1.6 ml, 0.022 mol) was added again, followed by n-butyl lithium (23 ml, 0.037 mol), keeping the temperature at -75°C. The mixture was stirred for 15 min. Each of the reagents, chloroiodomethane (0.70 ml, 0.010 mol) and n-butyl lithium (5 ml, 0.008 mol) were added 4 more times over 45 min. at -75°C. The cooling bath was then removed and the solution warmed to 22°C over 1.5 hr. The mixture was poured into 300 ml of saturated aq. ammonium chloride solution. The tetrahydrofuran layer was separated. The aqueous phase was extracted with ethyl acetate (1 x 300 ml). The combined organic layers were washed with brine, dried over magnesium sulfate, filtered and concentrated to give a brown oil (27.4 g). The product could be used in the next step without purification.

METHOD 3: A solution of 2S-[Bis(phenylmethyl)amino]-3-phenylpropanaldehyde (178.84 g, 0.54 mol) and bromochloromethane (46 mL, 0.71 mol) in tetrahydrofuran (1.8 L) was cooled to -30 to -35°C in a stainless steel reactor under a nitrogen atmosphere. A solution of n-butyl lithium in hexane (1.6 M, 340 mL, 0.54 mol) was then added at a rate that maintained the temperature below -25°C. After addition the mixture was stirred at -30 to -35°C for 10 minutes. More additions of reagents were carried out in the following manner: (1) additional bromochloromethane (14 mL) was added, followed by n-butyl lithium (102 mL) at < -25°C. After addition the mixture was stirred at -30 to -35°C for 10 minutes. This was repeated once. (2) Additional bromochloromethane (7 mL, 0.11 mol) was added, followed by n-butyl lithium (51 mL, 0.082 mol) at <-25°C. After addition the mixture was stirred at -30 to -35°C for 10 minutes. This was repeated 5 times. (3) Additional

bis(phenylmethyl)amino]-1-(2-methylpropyl)amino-4-phenylbutan-2(R)-ol in ethyl acetate (90 mL) over 15 minutes. The mixture was stirred at room temperature for about 2 hours. Solid was isolated by filtration, washed with ethyl acetate (2 x 20 mL) and dried in vacuo for about 1 hour to yield 21.86g of 97% diastereomerically pure salt. Mp = 174.99°C; Microanalysis: Calc.: C 71.05%, H 7.50%, N 5.53%; Found: C 71.71%, H 7.75%, N 5.39%.

Alternatively, crude 3(S)-[N,N-bis(phenylmethyl) amino]-1-(2-methylpropyl)amino-4-phenylbutan-2(R)-ol (5 g) was dissolved in methyl-tert-butylether (MTBE) (10 mL) and oxalic acid (1 g) in methanol (4 mL) was added. The mixture was stirred for about 2 hours. The resulting solid was filtered, washed with cold MTBE and dried to yield 2.1 g of white solid of about 98.9% diastereomerically pure (based on HPLC peak areas).

Part F: Preparation of 1-[N-[(1,3-benzodioxol-5-yl) sulfonyl]-N-(2-methylpropyl)amino]-3(S)-[N,N-bis(phenylmethyl)amino]-4-phenyl-2(R)-butanol

To N-[3(S)-[N,N-bis(phenylmethyl)amino]-2(R)-hydroxy-4-phenylbutyl]-N-isobutylamine•oxalic acid salt (354.7 g, 0.7 mole) in 1,4-dioxane (2000 mL) was added a solution of potassium carbonate (241.9 g, 1.75 moles) in water (250 mL). The mixture was stirred for 2 hours at room temperature followed by addition of 1,3-benzodioxole-5-sulfonyl chloride (162.2 g, 0.735 mole) in 1,4-dioxane (250 mL) over 15 minutes. The reaction mixture was stirred at room temperature for 18 hours. Ethyl acetate (1000 mL) and water (500 mL) were added and stirring continued for another 1 hour. The aqueous layer was separated and further extracted with ethyl acetate (200 mL). The combined ethyl acetate layers were washed with 25% brine solution (500 mL) and

bottle was placed in the hydrogenator and purged 5 times with nitrogen and 5 times with hydrogen. The reaction was allowed to proceed at 35°C with 63 PSI hydrogen pressure for 18 hours. Additional catalyst (125 mg) was added and, after purging, the hydrogenation continued for and additional 20 hours. The mixture was filtered through celite which was washed with methanol (2 X 10 mL). Approximately one third of the methanol was removed under reduced pressure. The remaining methanol was removed by aziotropic distillation with toluene at 80 torr. Toluene was added in 15, 10, 10 and 10 mL portions. The product crystallized from the mixture and was filtered and washed twice with 10 mL portions of toluene. The solid was dried at room temperature at 1 torr for 6 hours to yield the amine salt (4.5 g, 84%): m/z 421 [M+H]+.

Alternaively, to a THF solution of crude 1-[N-[(1,3-benzodioxol-5-yl)sulfonyl]-N-(2-methylpropyl)amino]-3(S)-[bis(phenylmethyl)amino]-4-phenyl-2(R)-butanol was added water (500 mL) followed by methanesulfonic acid (531g, 5.5 moles). The solution was stirred to insure complete mixing and added to a 5 gallon autoclave. Pearlman's catalyst (200g of 20% $Pd(OH)_2$ on C/ 50% water) was added to the autoclave with the aid of THF (500 mL). The reactor was purged four times with nitrogen and four times with hydrogen. The reactor was charged with 60 psig of hydrogen and stirring at 450 rpm started. After 16 hours, HPLC analysis indicated that a small amount of the mono-benzyl intermediate was still present. Additional catalyst (50g) was added and the reaction was allowed to run overnight. The solution was then filtered through celite (500g) to remove the catalyst and concentrated under vacuum in five portions. To each portion, toluene (500 mL) was added and removed under vacuum to azeotropically removed residual water. The resulting solid was divided into three portions

A solution of 312g of N-[2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl) propyl]-2S-[(phenylmethoxycarbonyl)amino]-3,3-dimethylbutanamide in 1L of tetrahydrofuran was hydrogenated in the presence of 100g of 4% palladium-on-carbon catalyst under 60 psig of hydrogen for 6 hours at room temperature. The catalyst was removed by filtration and the solvents removed under reduced pressure to afford 240g of the desired compound.

Part J: Preparation of N-[2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl) propyl]-2S-[(chloroacetyl)amino]-3,3-dimethylbutanamide

To a solution of 234.3 g (0.439 mol) of N-[2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl) propyl]-2S-amino-3,3-dimethylbutanamide in 1L of methylene chloride, was added 80 mL (59.5 g, 0.46 mol) of diisopropylethylamine, followed by the slow addition at room temperature of 78.8 g (0.46 mol) of chloroacetic anhydride while maintaining the temperature below 35°C. After stirring for an additional 1 hour, analysis by HPLC indicated a small amount of starting material was still present, and 1.5 g of chloroacetic anhydride was added. After 10 minutes, the solvents were removed under reduced pressure, 1 L ethyl acetate added, washed with 5% citric acid, saturated sodium bicarbonate, brine, dried over anhydrous magnesium sulfate, filtered and concentrated to yield 314 g of crude material. This was chromatographeed in 3 portions on a Prep 2000 Chromatogram on silica gel using 20-50% ethyl acetate/hexane to afford 165 g of the desired compound, m/e=616 (M+Li), 98% by HPLC.

Part K: Preparation of N-[2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)

Preparation of N-[2R-hydroxy-3-[(2-methylpropyl)[(1,3-benzodioxol-5-yl)sulfonyl]amino]-1S-(phenylmethyl) propyl]-2S-methyl-3-(methylsulfonyl)propanamide

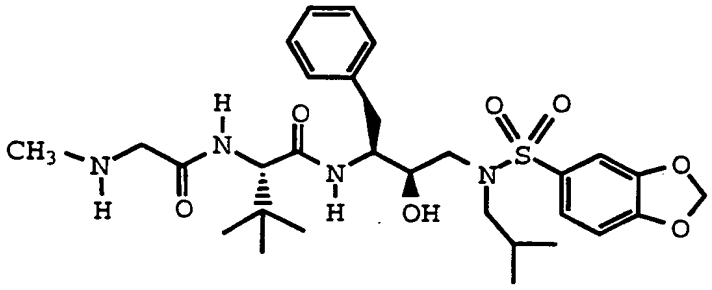
Part A: Preparation of 2(S)-methyl-3-(methylsulfonyl) propionic Acid

Step 1: To a solution of 200 g (1.23 mol) of D-(-)-3-acetyl-b-mercaptoisobutyric acid in 1.0 L of methanol, was added 161.0 g (2.47 mol) of potassium hydroxide dissolved in 500 mL of methanol while maintaining the temperature below 10°C while cooling with an ice bath. After stirring an additional 20 minutes, 117 mL (156 g, 1.23 mol) of dimethyl sulfate was added while maintaining the temperature below 20°C. The ice bath was removed and the mixture stirred for an additional 60 minutes. The salts were removed by filtration, the solvents removed under reduced pressure and ethyl acetate added. After separating the aqueous layer, it was acidified with concentrated hydrochloric acid, extracted with ethyl acetate, dried over anhydrous magnesium sulfate, filtered and concentrated to afford 164 g (99%) of the desired 2S-methyl-3-(methylthio)propionic acid, m/e = 133 (M-H).

Step 2: To a solution of 10.0 g (74.6 mmol) of 2S-methyl-3-(methylthio)propionic acid in 150 mL of acetone and 30 mL of water, cooled to 18°C in an ice bath, was added 161.8 g (263 mmol) of Oxone. After approximately half of material had been added, the temperature rose to 24°C, the addition was stopped, temperature lowered to 18°C, then addition continued. After stirring at 15-20°C for 15 minutes, the bath was removed and the reaction stirred at room temperature for 1 hour. The solids were filtered and washed with acetone, the filtrate concentrated to approximately 40 mL and the residue dissolved in 200 mL of ethyl acetate.

0°C under nitrogen, was added 44.4 g (231 mmol) of EDC. After approximately 30 minutes, the EDC was all dissolved. After an additional 60 minutes at 0°C, a solution of 108.8 g (211 mmol) of 2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propylamine methanesulfonate in 350 mL of anhydrous DMF, previouly neutralized with 24 mL (22.3g) of 4-methylmorpholine, was added. After 2 hrs at 0°C, the mixture was then stirred overnight for 18 hrs. The DMF was removed under reduced pressure, 1L of ethyl acetate added, washed with 5% citric acid, saturated sodium bicarbonate, water, brine, dried over anhydrous magnesium sulfate, filtered and concentrated to afford 120.4 g of crude material, which was 90% purity by HPLC. The product was crystallized twice from 750-1000mL of absolute ethanol to afford 82.6 g of the desired product.

Example 16



Preparation of 2S-[[(N-methylamino)acetyl]amino]-N-[2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]-3,3-dimethylbutanamide

To 6.55 g (10.7 mmol) of N-[2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]-2S-[(chloroacetyl)amino]-3,3-

time points (0, 8, 16 and 24 minutes) at ambient temperature. Each assay is carried out in duplicate wells.

Example 18

The effectiveness of selected HIV protease inhibitor compounds of the present invention can be determined using the above-described enzyme assay and the following CD4+ cell line assay. Antiviral activities of protease inhibitors are expressed as effective concentration 50 (EC_{50}) and/or effective concentration 90 (EC_{90}) values. These are the concentrations of inhibitors that were required to inhibit viral replication by 50% or 90%, respectively.

The HIV inhibition assay method of acutely infected cells is an automated tetrazolium based colorimetric assay essentially that reported by Pauwels et al, J. Virol. Methods 20, 309-321 (1988). Assays are performed in 96-well tissue culture plates. A CD4+ cell line, such as CEM, MT-2, MT-4 and the like cell lines, is grown in RPMI-1640 medium (Gibco) supplemented with a 10% fetal calf serum and are then treated with polybrene (2µg/ml). An 80 µl volume of medium containing 1×10^4 cells is dispensed into each well of the tissue culture plate. To each well is added a 100µl volume of test compound dissolved in tissue culture medium (or medium without test compound as a control) to achieve the desired final concentration and the cells are incubated at 37°C for 1 hour. A frozen culture of HIV-1 is diluted in culture medium to a concentration of 5×10^4 $TCID_{50}$ per ml ($TCID_{50}$ = the dose of virus that infects 50% of cells in tissue culture), and a 20 µL volume of the virus sample (containing 1000 $TCID_{50}$ of virus) is added to wells containing test compound and to wells containing only medium (infected control cells). Several wells receive culture medium without virus (uninfected control cells). Likewise, the intrinsic toxicity of the test compound is determined by

the test compound is determined by comparison of the absorbance values obtained in wells containing infected or uninfected cells incubated with compounds and the uninfected, untreated control wells.

HIV CULTURE PROCEDURES

STIMULATION OF DONOR LYMPHOCYTES

Buffy coats were obtained from the American Red Cross or Blood Bank at Washington University School of Medicine. These preparation are pre-screened for HIV and CMV antibodies and HBV surface antigen (HBsAg) and ALT (alanine transferase activity) as marker for non-A, non-B hepatitis. Leukocyte-enriched blood (30 ml) is removed from the plastic container and 15 ml is dispensed into two 50 ml screw-cap centrifuge tubes. Each sample is diluted with an equal volume of sterile PBS and mixed by pipeting. Ficoll-Paque (15 ml) or LSM is placed below the diluted blood samples using a Pasteur pipet and allowing the solution to drain to the bottom of the tube. Each of the tubes is then centrifuged at 1300 rpm (400xg) for 45 minutes at 20°C. Following centrifugation, the lymphocyte band at the interface is removed and transferred to a 50 ml tube. Sterile PBS is added to dilute the separated lymphocytes and then centrifuged at 1300 rpm for 8 minutes. The cell pellet is washed two times by resuspending in PBS and recentrifuging. The final cell pellet is resuspended in 20 ml PBS by pipetting and the total number of viable cells is determined by Trypan Blue exclusion.

ACUTE INFECTIVITY ASSAYS USING CLINICAL ISOLATES

Approximately $3x10^7$ cells are activated for 48 hours with about 3-5 µg/ml PHA in RPMI continuing 10% fetal bovine serum and IL-2 (10 U/ml). Quantitated virus stocks are added to the activated lymphocyte suspension at a

by electroblotting. HIV specific proteins are detected using monoclonal antibodies to p24 and p17 followed by goat-anti-mouse IgG linked to biotin, and avidin linked to HRP. Enzymatic conversion of 4-chloro-1-naphthol was used to visualize the specific proteins recognized by the monoclonal antibodies. In addition, the infectivity of virus produced by the chronically infected CEM cells in the presence or absence or the test compounds was examined. Filtered supernatants are serially diluted and used to infect uninfected CEM cells (about $1x10^4$/well). The cultures were examined for syncytia formation on days 7 and 11 post-infection or the supernants tested for reverse transcriptase activity or p24 antigen.

MICRO REVERSE TRANSCRIPTASE (RT) ASSAY

The micro RT assay is an adaptation of several standard RT assays. It was developed to allow small volume quantitative measurement of HIV RT activity and to facilitate processing of numerous samples.

MATERIALS	STOCK	WORKING SOLUTION (per 1 ml)
Tris (pH 7.8)	1.0 M	50µl
KCl	3.0 M	25µl
DTT (store -20°C)	0.1 M	20µl
$MgCl_2$	0.15 M	33µl
poly (rA)p(dT012-18) Pharmacia 27-7878	25 U/2.5ml	25µl (0.5 U)
NP-40	2%	25µl
^3H-TTP (NET 221A, 80 Ci/mmol)	2.5 Ci/ml	10µl
H_2O		777µl

The following is the culture method used for selection of HIV protease inhibitor resistant mutants. Infected cells were grown continually in the presence of protease inhibitor. Some cultures were subjected on alternate weeks to high and low inhibitor concentrations. Others were passaged in a constant concentration. Drug concentrations were increased periodically until a consistent shift in the EC_{50} was observed. A shift in the dose response curve was generally detected at drug concentration of 0.5 to 1 µg/ml or greater (5-10x the EC_{90}) and depended on the viral isolate being treated. Both laboratory adapted and primary clinical isolates of HIV were used. The same virus isolates were passaged in the same way in the absence of drugs so that direct nucleoside sequence comparisons could be made between treated and untreated isolates. Generally, HIV-1 variants resistant to protease inhibitors were select by serial passage (growth) in the presence of several inhibitory concentrations of the protease inhibitor specified (see Markowitz et al., Journal of Virology 69:701-706 (1995)). The HIV-1 variants listed below indicate the mutations which are present in the selected virus isolates and not present in the control, untreated virus isolates.

RF represents the HIV-1 strain $HIV\text{-}1_{RF}$ and RFR represents a mixture of resistant strains obtained by selection of RF against the compound of Example 1. RFR comprises a mixture of viral strains having protease genotypes of G48V (14/40 clones); G48V, V82A (18/40 clones); G48V, L90S (2/40 clones); G48V, I54T, V82A (1/40 clones); G48M (1/40 clones); G48V, Q61H (1/40 clones); V13I, G48V (1/40 clones); G48V, F53L, V82A (1/40 clones); and G48V, V82A, C95Y (1/40 clones). RFR2 represents a mixture of resistant strains obtained by cloning RFR by three rounds of growth at limiting dilution. RFR2 comprises a mixture of viral strains having protease genotypes of G48V, V82A (13/15 clones); G17E, G48V, V82A

after 37 passages comprising protease genotype M46I, L63P, A71V, I84A. NL4(538/524) represents resistant strains obtained by selection of NL4(P22-538) against the compound of Example 5 after 24 passages comprising protease genotype M46I, L63P, A71V, I84A. NL4(538/P7-AG) represents a mixture of resistant strains obtained by selection of NL4(P22-538) against the compound of Example 12 after 7 passages comprising protease genotypes M46I, L63P, A71V, I84A; and V32I, V82I. NL4(538/P24-AG) represents resistant strains obtained by selection of NL4(P22-538) against the compound of Example 12 after 24 passages comprising protease genotype M46I, L63P, A71V, I84A. NLA(P19-003) represents resistant strains obtained by selection of HIV-1_{NL4-3} against the compound of Example 9 after 19 passages comprising protease genotype R8K, M46I. NL4(P34-003) represents resistant strains obtained by selection of HIV-1_{NL4-3} against the compound of Example 9 after 34 passages comprising protease genotype R8K, M46I, L63P, A71V, L90M. Viral isolate resistance results are summarized in Tables 1-11.

Example 20

The viral isolate resistance results summarized in Tables 1-3 were generated according to the following assay procedure or minor modifications thereof. Approximately $3x10^7$ cells are activated for 48 hours with about 3-5 µg/ml PHA in RPMI continuing 10% fetal bovine serum and IL-2 (10 U/ml). Quantitated virus stocks are added to the activated lymphocyte suspension at a multiplicity of infection of about 0.001-0.01. The cell-virus suspension is incubated at 37°C for 2 hours to allow virus absorption. The residual virus inoculum is removed by centrifugation and the cells are resuspended in RPMI containing 10% FBS and 10 U/ml IL-2. These infected cells are added to the test-compound diluted in complete tissue culture medium from a stock (10 mg/ml) in

Example 21

The viral isolate resistance results summarized in Tables 4-6 were generated according to the following assay procedure or minor modifications thereof. Assays are performed in 96-well tissue culture plates. CEM-T4 cells are suspended in 90% RPMI media (Gibco BRL Life Technologies, Inc., Gaithsburg, MD) 10% heat-treated, fetal bovine serum (Gibco BRL Life Technologies, Inc., Gaithsburg, MD) to a final concentration of $5x10^5$ viable cells per ml. A frozen aliquot of an HIV culture (strain $HIV\text{-}1_{RF}$) is thawed rapidly (in a 37°C water bath) and added to the CEM-T4 cells to give a final concentration of about 0.001-0.01 infectious units per cell. The virus-cell suspension is rapidly mixed by swirling and 100 µL immediately added to 100 µL of each test-compound (prepared as a 2x concentrate in 90% RPMI, 10% FBS) dilution in each well of a 96-well tissue culture plate. Each plate contains control wells that comprise cells and virus but no test-compound. 3'-Azido-3'-deoxythymidine (AZT) is included as a positive control in all assays.

The tissue culture plates are incubated at 37°C in a humidified, 5% CO_2 atmosphere for 7 days. The level of viral replication is then determined by measurement of reverse transcriptase activity in the supernatents using standard methods (as previously described and see, for example, Techniques in HIV Research, Aldovini & Walker, eds., 1990, Stockton Press, NY).

Example 22

The viral isolate resistance results summarized in Tables 7-11 were generated according to the assay procedures described by Markowitz et al., Journal of Virology, vol. 69, 701-706 (1995), which is incorporated herein by reference in its entirety, or minor modifications thereof.

TABLE 7

Ex. No.	Virus Isolate (EC_{90} nM) NL4	NL4(G48V)	NL4(I84V)
5	80	160	640
6	30	150	90
10	80	160	800
12	25	125	125
13	250	1000	6250
14	8	8	72
15	60	60	60
16	8	8	8

TABLE 8

Ex. No.	Virus Isolate (EC_{50} nM) NL4	NL4(R8Q,M46I)
5	80	240
6	30	30
10	80	240
12	25	125
13	250	750
16	8	8

Example 23

Protease inhibitors of Examples 1 and 2, which contain a unique hydroxyethylurea isostere, were used to select drug resistant HIV-1 variants in vitro. Clinical and laboratory HIV-1 strains were passaged in T cell lines or peripheral blood mononuclear cells (PBMCs) in the presence of increasing drug concentrations. Resistant variants consistently exhibited EC_{50} values at least 10-fold higher than control virus passaged for an identical period, but in the absence of an inhibitor. Viral DNA was amplified by PCR and the nucleotide sequence of the gene encoding the protease was determined using standard methods. In viruses resistant to protease inhibitors of Examples 2 and 1, respectively, an amino acid change at position 88 was consistently observed in many of the variants selected. The Asn residue at 88 lies within a structurally conserved helical domain, present in both monomeric and dimeric aspartic proteinases. The corresponding carboxy terminal sequence Gly-Arg-Asp/Asn (residues 86-88) is unique to retroviral aspartic proteinases. While any explanation for these results is only speculation, modeling studies based on templates derived from high resolution x-ray structures of prototypical hydroxyethylurea inhibitors bound to recombinant HIV-1 protease appear to suggest that the Asn88 mutations may alter the conformation of the protease.

Retroviral protease inhibitor compounds of the present invention are advantageously effective antiviral compounds and, in particular, are effective inhibitors of retroviruses, particularly, lentiviruses as shown above. Thus, the subject compounds are effective inhibitors of HIV. It is contemplated that the subject compounds will also inhibit other strains of HIV, such as HIV-2 and other viruses such as, for example, VISNA virus and Simian

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

The dosage regimen for treating a disease condition with the retroviral protease inhibitor compounds and/or compositions is selected in accordance with a variety of factors, including the type, age, weight, sex, diet and medical condition of the patient, the severity of the disease, the route of administration, pharmacological consideration such as the activity, efficacy, pharmacokinetic and toxicology profiles of the particular compound employed, whether a drug delivery system is utilized and whether the compound is administered as part of a drug combination. Thus, the dosage regimen actually employed may vary widely and therefore may deviate from the preferred dosage regimen set forth above.

The compounds of the present invention may be administered orally, parenterally, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired. Topical administration may also involve the use of transdermal administration such as transdermal patches or iontophoresis devices. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection, or infusion techniques.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable siluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents

not limited to, other HIV-1 protease inhibitors, various nucleoside analogs, nonnucleoside reverse transcriptase inhibitors, tat antagonists and glycosidase inhibitors.

Examples of HIV-1 protease inhibitors include, but not limited to, Ro 31-859 (Roberts, N.A. et al. Science 1990, 248, 258-261 and Drugs of the Future 1991, 16(3), 210-212, KNI-272, (Kagayama, S., et al. Antimicrobial Agents and Chemotherapy 1993, 810-817), the cyclic urea series (Lam, P., et al., "De Novo Design and Discovery of Potent, Nonpeptidal HIV-1 Protease Inhibitors," paper 96 at the 205th American Chemical Society National Meeting, Medicinal Chemistry Division, Denver, CO, March 28-April 2, 1993), L-735,524 (Dorsey, B.D., et al., "L-735,524: The Rational Design of a Potent and Orally Bioavailable HIV Protease Inhibitor," paper 6 at the 206th American Chemical Society National Meeting, Medicinal Chemistry Division, Chicago, IL, August 22-27, 1993) and analogs thereof.

Examples of competitive nucleoside analogs include, but are not limited to, azidothymidine (AZT), dideoxyinosine (DDI), DDC, 3TC, D4T and PMEA. Examples of non-nucleoside, non-competitive reverse transcriptase inhibitors include, but are not limited to, the pyridone class (Wei, J.S., et al. J. Med. Chem. 1993, 36, 249-255; Hoffman, J.M., et al. J. Med. Chem. 1992, 35, 3784-3791; Saari et al. J. Med. Chem. 1992, 35 3792-3802; Drugs of the Future 1992, 17(4), 283-285, and analogs thereof); the bis-(heteroaryl)piperazines class (Romero, D.L., et al. J. Med. Chem. 1993, 36, 1505-1508; Romero, D.L., et al. Proc. Natl. Acad. Sci. USA 1991, 34, 746-751 and 3187-3198; and analogs thereof) and the tricyclic pyridobenzo- and depyridodiazepinones (Hargrave, K.D., J. Med. Chem. 1991, 34, 2231-2241; Merluzzi, M.J. Science 1990, 250, 1411-1413; and analogs thereof) and 5-chloro-3-(phenylsulfonyl)indole-2-carboxamide and its analogs (Williams, T.M. et al., J. Med. Chem. 1993, 36, 1291-1294). Examples of tat

calibrators and controls. Prior to and during the growth and storage of a cell culture, the subject compounds may be added to the cell culture medium at an effective concentration to prevent the unexpected or undesired replication of a retrovirus that may inadvertently or unknowingly be present in the cell culture. The virus may be present originally in the cell culture, for example HIV is known to be present in human T-lymphocytes long before it is detectable in blood, or through exposure to the virus. This use of the subject compounds and methods prevent the unknowing or inadvertent exposure of a potentially lethal retrovirus to a researcher or clinician.

The foregoing is merely illustrative of the invention and is not intended to limit the invention to the disclosed compounds. Variations and changes which are obvious to one skilled in the art are intended to be within the scope and nature of the invention which are defined in the appended claims.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

[2R-hydroxy-3-[[(4-aminophenyl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]carbamic acid 3S-tetrahydrofuranyl ester;

N-tert-Butyl decahydro-2-[2(R)-hydroxy-4-(phenylthio)-3(S)-[[N-[(2-methyl-3-hydroxyphenyl)carbonyl]amino]butyl]-(4aR,8aS)-isoquinoline-3(S)-carboxamide;

[4R-(4α,5α,6ß,7ß)]-1,3-bis[(3-aminophenyl)methyl]hexahydro-5,6-dihydroxy-4,7-bis(phenylmethyl)-2H-1,3-diazepin-2-one;

N-[2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]-2S-[[(pyrrolidin-1-yl)acetyl]amino]-3,3-dimethylbutanamide;

N-[2R-hydroxy-3-[(2-methylpropyl)[(1,3-benzodioxol-5-yl)sulfonyl]amino]-1S-(phenylmethyl)propyl]-2S-methyl-3-(methylsulfonyl)propanamide;

[1S-[1R*(R*),2S*]]-N-[2-hydroxy-3-[N^1-(2-methylpropyl)-N^1-(4-methoxyphenylsulfonyl)amino]-1-(phenylmethyl)propyl]-2-methyl-3-(methylsulfonyl)propanamide;

2S-[[(N-methylamino)acetyl]amino]-N-[2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]-3,3-dimethylbutanamide; or

(2R,3S)-3-(N-methylaminoacetyl-L-tert-butylglycinyl)amino-1-(N-isoamyl-N-(tert-butylcarbamoyl))amino-4-phenyl-2-butanol.

5. Method of Claim 1 wherein said second retroviral protease inhibitor is

N-(2(R)-Hydroxy-1(S)-indanyl)-2(R)-phenylmethyl-4(S)-hydroxy-5-(1-(4-(3-pyridylmethyl)-2(S)-N'-(t-butylcarboxamido)-piperazinyl))-pentaneamide;

N-tert-Butyl decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparaginyl]amino]butyl]-(4aR,8aS)-isoquinoline-3(S)-carboxamide;

(2S,3R,4S,5S)-2,5-Bis-[N-[N-[[N-methyl-N-(2-pyridinylmethyl)amino]carbonyl]valinyl]amino]-3,4-dihydroxy-1,6-diphenylhexane;

(2S,3R,4S,5S)-2,5-Bis-[N-[N-[[N-methyl-N-(2-pyridinylmethyl)amino]carbonyl]valinyl]amino]-3,4-dihydroxy-1,6-diphenylhexane;

(2S,3S,5S)-5-[N-[N-[N-methyl-N-[(2-isopropyl-4-thiazolyl)methyl]amino]carbonyl]valinyl]amino]-2-[N-[(5-thiazolyl)methoxycarbonyl]amino]-3-hydroxy-1,6-diphenylhexane;

[2R-hydroxy-3-[[(4-aminophenyl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]carbamic acid 3S-tetrahydrofuranyl ester;

N-tert-Butyl decahydro-2-[2(R)-hydroxy-4-(phenylthio)-3(S)-[[N-[(2-methyl-3-hydroxyphenyl)carbonyl]amino]butyl]-(4aR,8aS)-isoquinoline-3(S)-carboxamide;

[4R-(4α,5α,6ß,7ß)]-1,3-bis[(3-aminophenyl)methyl]hexahydro-5,6-dihydroxy-4,7-bis(phenylmethyl)-2H-1,3-diazepin-2-one;

N-[2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]-2S-[[(pyrrolidin-1-yl)acetyl]amino]-3,3-dimethylbutanamide;

N-[2R-hydroxy-3-[(2-methylpropyl)[(1,3-benzodioxol-5-yl)sulfonyl]amino]-1S-(phenylmethyl)propyl]-2S-methyl-3-(methylsulfonyl)propanamide;

[1S-[1R*(R*),2S*]]-N-[2-hydroxy-3-[N^1-(2-methylpropyl)-N^1-(4-methoxyphenylsulfonyl)amino]-1-(phenylmethyl)propyl]-2-methyl-3-(methylsulfonyl)propanamide; or

(2R,3S)-3-(N-methylaminoacetyl-L-tert-butylglycinyl)amino-1-(N-isoamyl-N-(tert-butylcarbamoyl))amino-4-phenyl-2-butanol; and

said second retroviral protease inhibitor is

2S-[[(N-methylamino)acetyl]amino]-N-[2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]-3,3-dimethylbutanamide.

7. Method of Claim 1 wherein said first retroviral protease inhibitor is

9. Method of Claim 1 wherein a third retroviral protease inhibitor is administered to said patient, wherein said third retroviral protease inhibitor is effective against at least one viral strain that is resistant to both said first and second retroviral protease inhibitors.

10. Method of Claim 9 wherein said third retroviral protease inhibitor is

N-(2(R)-Hydroxy-1(S)-indanyl)-2(R)-phenylmethyl-4(S)-hydroxy-5-(1-(4-(3-pyridylmethyl)-2(S)-N'-(t-butylcarboxamido)-piperazinyl))-pentaneamide;

N-tert-Butyl decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparaginyl]amino]butyl]-(4aR,8aS)-isoquinoline-3(S)-carboxamide;

(2S,3R,4S,5S)-2,5-Bis-[N-[N-[[N-methyl-N-(2-pyridinylmethyl)amino]carbonyl]valinyl]amino]-3,4-dihydroxy-1,6-diphenylhexane;

(2S,3S,5S)-5-[N-[N-[N-methyl-N-[(2-isopropyl-4-thiazolyl)methyl]amino]carbonyl]valinyl]amino]-2-[N-[(5-thiazolyl)methoxycarbonyl]amino]-3-hydroxy-1,6-diphenylhexane;

[2R-hydroxy-3-[[(4-aminophenyl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]carbamic acid 3S-tetrahydrofuranyl ester;

N-tert-Butyl decahydro-2-[2(R)-hydroxy-4-(phenylthio)-3(S)-[[N-[(2-methyl-3-hydroxyphenyl)carbonyl]amino]butyl]-(4aR,8aS)-isoquinoline-3(S)-carboxamide;

[4R-(4α,5α,6ß,7ß)]-1,3-bis[(3-aminophenyl)methyl]hexahydro-5,6-dihydroxy-4,7-bis(phenylmethyl)-2H-1,3-diazepin-2-one;

N-[2R-hydroxy-3-[[(1,3-benzodioxol-5-yl)sulfonyl](2-methylpropyl)amino]-1S-(phenylmethyl)propyl]-2S-[[(pyrrolidin-1-yl)acetyl]amino]-3,3-dimethylbutanamide;

N-[2R-hydroxy-3-[(2-methylpropyl)[(1,3-benzodioxol-5-yl)sulfonyl]amino]-1S-(phenylmethyl)propyl]-2S-methyl-3-(methylsulfonyl)propanamide;

17. Method of Claim 16 wherein said retrovirus is HIV-1 or HIV-2.

18. Method of treating retroviral infections in a patient comprising:

(a) selecting two retroviral protease inhibitors wherein a second selected retroviral protease inhibitor is effective against at least one retroviral strain that is resistant to a first selected retroviral protease inhibitor; and

(b) administering to said patient an effective amount of each said first and second selected inhibitors.

19. Method of Claim 18 wherein said first and second inhibitors are administered such that an effective amount of both inhibitors are present in said patient.

20. Method of Claim 18 wherein the administration of each said first and second inhibitors is alternated such that an effective amount of one inhibitor at a time is present in said patient.